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Assessment of RNA carrier function in peptide amphiphiles derived from the HIV fusion peptide

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ABSTRACT

A small library of amphiphilic peptides has been evaluated for duplex RNA carrier function into A549 cells. We studied peptides in which a *C*-terminal 7-residue cationic domain is attached to a neutral/hydrophobic 23-residue domain that is based on the viral fusion peptide of HIV. We also examined peptides in which the cationic charge was evenly distributed throughout the peptide. Strikingly, subtle sequence variations in the hydrophobic domain that do not alter net hydrophobicity result in wide variation in RNA uptake. Additionally, cyclic cystine variants are much less active as RNA carriers than their open-chain cysteine analogs. With regard to electrostatic effects, we find that lysine is less effective than arginine in facilitating uptake, and that even distribution of cationic residues throughout the peptide sequence results in especially effective RNA carrier function. Overall, minor changes in peptide hydrophobicity, flexibility and charge distribution can significantly alter carrier function. We hypothesize this is due to altered properties of the peptide-RNA assembly rather than peptide secondary structure.

1. Introduction

Synthetic and natively derived peptide carriers for nucleic acid delivery have been the subject of many studies [1,2]. The relationship between membrane activity of peptides against synthetic lipid vesicles [3–5] and biomembrane activity remains unpredictable [6–8]. We have previously reported on the synthetic vesicle membrane fusion [9–14] and lysis activity of a library of HIV fusion peptide variants [15]. One major goal of this prior study was to understand the origins of membrane activity within the framework of a viral fusion peptide. To probe the biophysical function of the monomeric form of these hydrophobic peptides, each variant of the 23-residue fusion peptide domain was synthesized with a *C*-terminal cationic heptapeptide to promote solubility [16,17]. Interestingly, we found considerable variation in membrane activity upon shuffling the amino acid sequence of the HIV fusion peptide; moreover, within this 30-residue diblock peptide amphiphile architecture it was possible to identify variants that were purely fusogenic or lytic against negatively charged synthetic lipid vesicle membranes [15]. Though derived from native viral sequences, the separation of hydrophobic and polar residues into two distinct, fused peptide domains is more akin to polymer carriers [18–20] than native transduction domains [21–23]. Herein, we describe our studies on the function of these peptide amphiphiles as carriers for duplex RNA cargo across cell membranes. The cationic domain of each peptide, previously used to facilitate binding to anionic membranes [15], was used in this study to electrostatically bind fluorescein-labeled 21 nt duplex RNA (Fig. 1). We found that the peptide-RNA complexes could be taken up into cells in culture without additional transfection agent, as judged by FACS. However, it was much more difficult to determine a clear relationship between sequence and function. Sequences that displayed strong fusogenic membrane activity against synthetic vesicles were not necessarily effective RNA transporters into A549 cells in culture; indeed, no clear correlation could be found between vesicle data and cell uptake data (Table 1). Furthermore, while a strongly lytic peptide with evenly distributed cationic residues was highly effective in RNA transport, a similarly patterned peptide with a different sequence by nearly identical overall hydrophobicity was a totally ineffective RNA carrier. The sequence dependence of peptide-RNA transport across biomembranes has proven to be considerably more difficult than examination of membrane activity of synthetic vesicles. Generally, uptake is indeed strongly sensitive to sequence variation and depends less on overall hydrophobicity. Despite the poor correlation between synthetic membrane activity and RNA cellular delivery, we find broad guidelines for improving carrier function in the peptide diblock amphiphile architecture.

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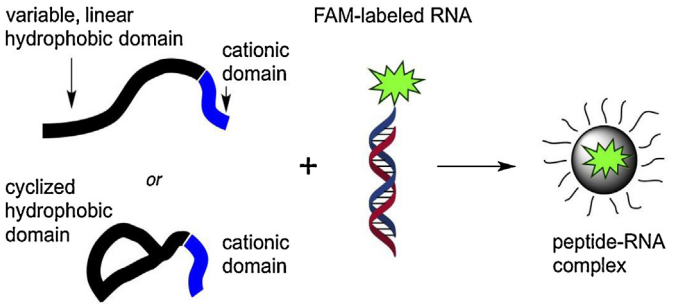

Fig. 1. Illustration of peptide diblock architecture and electrostatic complexation with fluorescein-labeled duplex RNA.

Table 1
Peptide sequences and uptake activity.

#	HIV fusion peptide sequence	% uptake	
1	AVGIGALFLGFLGAAGSTMGARS-R_6W	29	
1K	AVGIGALFLGFLGAAGSTMGARS-K_6W	6	
	Glycine shuffled peptides		
2	GAVIGALGFLFGLAAGSTMGARS-R_6W	34	
3	AVIGALFGLFLGAASGTMAGRSG-R_6W	40	
4	AVIALFLGGGFLAASGGGTMARS-R_6W	18	
5	AVIGGALFLGGGFLGAASTMARS-R_6W	38	
6	AVIGALFGLFGGLAASGTMGARS-R_6W	44	
7	AVIGGGALFLGGFLAASGTMARS-R_6W	47	
8	AVIGGALFLGGFLGAASGTMARS-R_6W	51	
9	AVIAGGLFLFGGLAASGGTMARS-R_6W	40	
10	AVIGGALFLGFLGAASGGTMARS-R_6W	54	
11	AVIALFLGGGGGGFLAASTMARS-R_6W	12	
12	AVIGGALFLGGFLAASGGTMARS-R_6W	56	
	Electrostatic effects		
13	AVGRIGARLFLRGFLRGAARGSTRMGARSW	70	
14	ALVRGGARLVLRGGVRLAARAGGRVLARSW	1	
15	RSRRRRRRW	0.3	
	Cysteine/cystine	-SH)$_2$	S-S
26	ALVGGALCLGGCLAAAGGVLARS-R_6W	54	4
27	ALCGGALCLGGVLAAAGGVLARS-R_6W	39	4
28	ALCGGALVLGGCLAAAGGVLARS-R_6W	33	1
29	ALVGGALVLGGCLAAAGGCLARS-R_6W	27	6
30	ALCGGALVLGGVLAAAGGCLARS-R_6W	63	5

2. Materials and methods

2.1. General

All siRNA and 5X siRNA buffer were purchased from Thermo Scientific (Dharmacon). Phenol Red-free DMEM, FBS, Penicillin-Streptomycin, 0.05% Trypsin-EDTA (1X), 1X DPBS, Hoechst 33258 and Opti-MEM were purchased from Invitrogen. Heparin sodium salt (from porcine intestinal mucosa) and Fluoroshield^{TM} (mounting medium) were purchased from Sigma Aldrich.

2.2. Peptide synthesis

Peptides were synthesized using standard Fmoc solid phase peptide chemistry (Rink LS resin) on an Apex 396 AAPPTec peptide synthesizer and purified to homogeneity by RP-HPLC, as described previously [15]. Briefly, diisopropyl carbodiimide (DIC)/HOBt was used as a coupling reagent with 1-hour standard coupling time. Peptides were cleaved from resin using a mixture of TFA: thioanisole: m-cresol: ethanedithiol: H_2O in the ratio of 87.5:5:2.5:2.5:2.5 for 3.5 h. Peptides were precipitated from the cleavage cocktail with cold diethyl ether and purified by reversed-phase HPLC (C_{18}) on water-acetonitrile (0.1% TFA) gradients. Stock solutions of peptides were prepared in deionized H_2O and stored at −20 °C. The concentration of peptides were determined by the UV-absorbance (Trp) at 280 nm ($\varepsilon = 5502\ M^{-1}cm^{-1}$).

2.3. Nucleic acids

Duplex FAM-labeled RNA was purchased from Dharmacon: 5′-(FAM)-CUUACGCUGAGUACUUCGA-dTdT-3′ and 5′-UCGA-AGUACUCAGCGUAAG-dTdT-3′.

2.4. RNA uptake

A549 cells were seeded in 12 well plates at 150,000 cells/well and incubated in growth medium containing 90% phenol red-free DMEM, 10% RBS, 100 units/ml penicillin, 100 μg/ml streptomycin at 37 °C, and 5% CO_2 and grown for 24 h. Prior to transfection, media was removed and 1 ml of fresh DMEM added to each well. Cells were then transfected with 10, 20, and 40 pmol FAM-GL3 siRNA duplex complexed with peptides at N/P (Nitrogen-based cation to Phosphorous-based anion) ratios of 1–15 to determine the optimal condition for transfection. At RNA concentrations 8 nM and 17 nM, the transfection efficiency was low (less than 10% positive cells) and could not be improved by increasing N/P ratio. A significant increase in uptake was observed at 33 nM RNA, improved by increasing N/P ratio from 5 to 10, with maximum uptake at N/P = 10 and decreasing efficiency at N/P > 10. Uptake experiments were thus performed with N/P = 10 at 33 nM total RNA.

Transfections were performed in triplicate. RNA/peptide complexes were prepared by incubation of 120 μl of peptide in Opti-MEM with 120 μl of 48 pmol RNA for 1 h. 200 μl of the RNA/peptide complex solution was added to cells in each well and incubated 6 h at 37 °C, 5% CO_2. The medium was then removed, and cells were incubated in fresh media for another 6 h at 37 °C, 5% CO_2. Cells were then washed with DPBS (3×) and heparin solution (15 units/ml, 3×) three times Cells were incubated with heparin solution for 20 min at 37 °C, 5% CO_2 for each wash. After heparin treatment, cells were again washed with 1X DPBS and trypsinized (37 °C, 10–15 min), resuspended in 700 μl media and spun down and washed with DPBS. Cells were resuspended and fixed in 4% paraformaldehyde (4 °C, 10 min), washed in DPBS (2×) and analyzed by a LSR-II flow cytometer gated on FAM fluorescence to determine %positive cells on volume of 10,000 cells.

3. Results and discussion

A small peptide library was prepared using solid phase peptide synthesis as described previously [15] and examined for RNA carrier function. Each peptide was complexed with fluorescein-labeled 21 nt duplex RNA. Like known RNA carriers such as lipofectamine, the peptide-RNA complexes underwent higher order assembly into a sample of particles of heterogeneous distribution, as judged by dynamic light scattering. The polydispersity of the particle assembly precludes reliable assessment of particle size, though they appear to be in the range of hundreds of nanometers to microns. RNA carrier function against A549 cells was found to vary significantly with sequence, with no clear functional relationship between sequence and function, though general trends could be observed. This is perhaps not surprising, given the greater complexity of a biomembrane relative to a synthetic vesicle and the variability scattering sizes found in RNA-peptide complexation.

A subset of 12 peptides with distinct hydrophobic and cationic domains was studied wherein the hydrophobic domains had amino acid compositions identical to that of the 23-residue HIV fusion peptide. This viral fusion domain has 6 glycines, which are thought to be essential to viral infectivity [24,25]. To probe the importance of these residues on RNA transport, the glycines were “shuffled”